REMARKS

Courtesies extended to Applicant and Applicant's representatives during the telephone interview held on April 4, 2006, are acknowledged with appreciation. Applicant respectfully requests reconsideration of the present application in view of the claim amendments and remarks that follow.

As discussed during the telephone interview, the present invention provides methods for identifying active proteins in a complex protein mixture (*e.g.*, a proteomic mixture). Complex protein mixtures are reacted with a single activity based probe (ABP—preferably an ABP that binds to active target proteins at a single site), and the resulting protein conjugates are proteolytically digested, providing probe-labeled peptides. (See Exhibit I). Prior to the present invention (as reflected by the prior art cited by the Examiner, i.e., Aebersold and Cravatt; see also Exhibits 2-4), the skilled artisan would have expected that proteolytic digestion would result in a more complex protein mixture. In contrast, the present invention demonstrates that such proteolysis simplifies the complex protein mixture during subsequent analysis.

In preferred embodiments, ABPs are selected such that each active target protein forms a conjugate with a single ABP, preferably at a single discrete location in the target protein; thus, each conjugate gives rise to a single peptide labeled with a single probe. Enrichment separation or identification of one or more ABP-labeled peptides may be achieved using liquid chromatography and/or electrophoresis. Mass spectrometry may be employed to identify one or more ABP-labeled peptides by molecular weight and/or amino acid sequence. In particularly preferred embodiments, sequence information derived from one or more of the ABP-labeled peptide(s) may be used to identify the protein from which the peptide was originally derived.

Thus, methods of the present invention provide enhanced simplicity and accuracy in the identification of the active protein composition of a complex protein mixture.

By the present communication, claims 21, 49, 60 and 74 have been amended to define Applicant's invention with greater particularity. No new matter is introduced by the subject amendments as the amended claim language is fully supported by the specification and the original claims. Claims 60-73 have been withdrawn from consideration. Accordingly, claims 21-32 and 48-74 remain pending, with claims 21-32, 48-59 and 74 under active prosecution. The present status of all claims in the application is indicated in the Listing of Claims, which begins on page 2.

In the Amendment submitted herewith, claims 21, 49 and 74 have been amended in accordance with the suggestion of Supervisory Examiner Li during the telephone interview, to define the invention with greater particularity. Specifically, Supervisor Li suggested that the transitional phrase "consisting essentially of" be replaced with "consisting of", and that the claim be further amended to explicitly demonstrate the order of method steps. Withdrawn claim 60 has been similarly amended to include the transitional phrase "consisting of." Rejoinder of claim 60, and dependent claims 61-73 is respectfully requested.

The Examiner has asserted that where there is no clear indication of the basic and novel characteristics of the invention, the transitional phrase "consisting essentially of" may be construed as "comprising." (See page 7, line 20 to page 8, line 4 of the Office Action dated January 19, 2006). Applicant respectfully disagrees with the Examiner's assertion. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristics of the claimed invention, as a "consisting essentially of" claim occupies a middle ground between closed claims that are written in a "consisting of" format and fully open claims that are drafted in a "comprising" format. See MPEP §2111.03, and references cited therein. As clearly shown by the original claims, the specification (as shown, for example, in paragraphs [0011] and [0022]), and the Exhibits submitted herewith, the novel characteristics of the claims, as currently amended, include the use of activity based probes and the specific sequential steps required by

the claim. However, without agreeing with the Examiner, and in order to reduce the issues and expedite prosecution of the present application, Applicant has amended each of claims 21, 49, 60 and 74 to include the transitional phrase "consisting of."

The rejection of claims 21-28, 30-32, 48-54, 56-59 and 74 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Aebersold et al., U.S. 2002/0076739, in view of Cravatt et al., U.S. 2002/0045194, is respectfully traversed. As discussed in the prior response (dated October 28, 2006) Applicant's invention distinguishes over the combination of Aebersold in view of Cravatt in at least the following ways:

(1) the combination of Aebersold and Cravatt does not teach the use of a single activity based probe wherein digestion of the protein sample is done prior to separation;
(2) the prior art contemplates the use of sets of isotopically labeled probes, rather than the use of a single activity based probe, as required by the present invention; and
(3) prior to the present invention, the belief in the art was that a single peptide would not provide accurate identification of proteins.

Specifically, Applicant's invention, as defined, for example, by claim 21 and shown in Exhibit I, distinguishes over Aebersold, in view of Cravatt, by requiring a method for determining the presence, amount, or activity of one or more active target proteins in a complex protein mixture, the method consisting of the following sequential steps:

(a) contacting said complex protein mixture with a single activity based probe that specifically binds predominantly to a single target site on one or more active target proteins;

(b) optionally binding said target protein(s) to a solid support;

(c) proteolyzing said active target protein(s) to produce a product mixture;

(d) separating said product mixture into two or more components, one or more of which consist essentially of peptides bound to said probe; and thereafter

(e) generating a signal from said peptides bound to said probe, wherein said signal is correlated to the presence, amount, or activity of said one or more active target proteins in said complex protein mixture.

As discussed at length during the telephone interview, neither Aebersold nor Cravatt, individually or in combination, teach such a method for determining the presence, amount or activity of one or more active target proteins employing an activity based probe following the specific sequential steps provided in the claim.

In the Office Action dated January 19, 2006, the Examiner acknowledged that Aebersold fails to teach the use of an activity based probe and the use of a single activity based probe. In efforts to cure this deficiency, the Examiner relies upon Cravatt, alleging that the combination of Cravatt and Aebersold teach all of the elements of the invention, as claimed. However, the Examiner's assertion is inaccurate because Cravatt does not contemplate a method for analyzing proteins in a single complex protein mixture, e.g. a proteome, wherein each element of the present invention, as required for example in claim 21, is described. As currently amended, claim 21 requires that the method steps be employed in the order specified in the claim, i.e., protein digestion is done after separation of the product mixture. In contrast, Cravatt teaches that when a single activity based probe is used to label a protein sample, protein digestion is done after separation. See Cravatt paragraphs [0107] and [0183-0184]; see also Exhibit III. Cravatt only contemplates protein digestion prior to separation when two or more protein samples are quantitatively compared using sets of isotopically labeled activity based probes. See Cravatt paragraph [0128]; see also Exhibit IV. Thus, the present invention is clearly distinct from the Cravatt method.

No combination of Aebersold and Cravatt suggests or discloses every element of the inventive methods in the order specified in the present claims. The Cravatt method is the result of combining the Aebersold method and the use of activity based probes (see Cravatt paragraph

[0128]), wherein two samples are compared using two isotopically distinct activity based probes, and the resulting isotopically labeled peptides are analyzed using mass spectrometry. While the Cravatt method employs protein digestion prior to separation, the remaining method steps are clearly different from the steps required by the present claims. Specifically, Cravatt discloses the use of isotopically labeled sets of probes. In contrast, the present invention employs single activity based probes.

Moreover, Applicant respectfully disagrees with the Examiner's assertion that:

> It would have been obvious to one of ordinary skill in the art to substitute the activity based probe such as taught by Cravatt et al for the probe of Aebersold et al because Cravatt et al teaches the use of a single probe in single and combined samples and also because Cravatt et al recognized the need for methods of measuring protein activity in proteomics. . . Therefore, a skilled artisan can have a reasonable expectation of success in incorporating an activity based probe taught by Cravatt et al in the method of Aebersold et al.

(See page 4, lines 17 to page 5, line 5 of the Office Action; emphasis added.) Contrary to the Examiner's assertion, recognition of a need in the art is not the same as an expectation of success. Mere recognition of a need may provide the motivation to try, but it clearly does not, without more, rise to the level of an expectation of success. The Examiner provides absolutely no support from either Aebersold or Cravatt for the assertion that a skilled artisan would have an expectation of success in the combination of the Cravatt and Aebersold methods. Furthermore, as discussed above, the Cravatt method is the result of combining the Aebersold method with activity based probes, wherein two samples are compared using isotopically labeled activity based probes. (See Exhibit IV). The claims as currently amended are clearly not a combination of Aebersold and Cravatt (i.e., activity based probes), because the Aebersold method requires the use of a different probe (i.e., sets of isotopically distinct probes) for a different purpose (i.e., quantitative comparison of multiple samples).

Applicant also respectfully disagrees with the Examiner's assertion that "applicant is arguing the references individually." (See page 9, lines 2-3 of the Office Action dated October 28, 2005; and again raised by the Examiner during the telephone interview). It is only by looking at the teaching of each reference individually that one can evaluate whether the asserted combination of references is logical, and if so, what a logical combination of the teachings would actually suggest to one of skill in the art.

As discussed previously, labeling with the activity based probes contemplated for use in the methods of the present invention produce fewer peptides than the use of the Aebersold probes. Contrary to the Examiner's assertion, the reason fewer peptides are produced by the present method is not because the present invention employs a single activity based probe and Aebersold employs multiple probes. Instead, fewer peptides are produced because activity based probes, as defined in the present invention, react with a specific amino acid side chain only when it is within a particular structural/functional context, i.e., an enzyme active site. For example, while the amino acid sequence of a hydrolase enzyme may have multiple occurrences of a particular residue, the activity based probe of the present invention will only react with and label the single residue which is part of the enzyme active site. In contrast, the Aebersold probes, when similarly applied, will label every occurrence of the particular residue present in the protein.

As discussed in detail during the telephone interview, and in Applicant's previous responses (see Responses dated October 28, 2005 and April 26, 2005), Aebersold does not disclose or suggest a method for determining the presence, amount, or activity of one or more active target proteins in a complex protein mixture. As acknowledged by the Examiner, "Aebersold et. al. differ from the instant invention in failing to teach the probe is an activity based probe and the use of a single activity based probe." (See page 4, lines 1-2 of the Office Action). Thus, the Aebersold method (i.e., using sets of isotopically labeled probes) is distinct from the claimed invention in at least two significant ways: Aebersold discloses the use of a set

(as opposed to a single probe) of isotopically labeled probes (as opposed to an activity based probe) for the quantitative comparison of protein samples. (See Exhibit II).

In contrast to Aebersold, the use of activity based probes according to the present invention results in the labeling of a single site on each target; and subsequent proteolytic digestion does not increase the complexity of the sample. Each probe employed in the Aebersold method labels multiple sites on the target proteins because the Aebersold probes are generally reactive with a particular amino acid side chain (e.g., cysteine), and lack specificity for the context of the residue within the structure/function of the protein. In contrast, the activity based probes of the present invention are reactive with a specific amino acid side chain only when it is within a particular structural/functional context (i.e., an enzyme active site). The Examiner's attention is directed to the responses dated October 28, 2005 and April 26, 2005 for a full discussion of the impact of the use of generally reactive probes as compared with the use of activity based probes.

In view of the above background discussion providing context for the work described by Aebersold, it is respectfully submitted that reliance on Cravatt does not cure the deficiencies of Aebersold. The combination of Aebersold and Cravatt does not teach every element of the invention according to the specified steps and specific order (i.e., the quantitative analysis of multiple proteins in a sample with a single activity based probe wherein protein digestion precedes separation), as required by the amended claims.

Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) as allegedly being unpatentable over Aebersold et al. in view of Cravatt are respectfully requested.

The rejection of claims 29 and 55 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Aebersold et al. and Cravatt, and further in view of Little et al., U.S. 2003/0003465, is respectfully traversed. Applicant's invention, as defined, for example, by claim 29, distinguishes over the combination of Aebersold and Cravatt in view of Little, by

requiring a method for determining the presence, amount, or activity of one or more active target proteins in a complex protein mixture, the method consisting of:

(a) contacting the complex protein mixture with a single activity based probe that specifically binds predominantly to a single target site on one or more active target proteins;

(b) optionally binding said target protein(s) to a solid support;

(c) proteolyzing the active target protein(s) to produce a product mixture, wherein prior to proteolyzing, the one or more active target proteins bound to the probe are bound to a solid support;

(d) separating the product mixture into two or more components, one or more of which consist essentially of peptides bound to the probe; and thereafter

(e) generating a signal from the peptides bound to the probe, wherein the signal is correlated to the presence, amount, or activity of the one or more active target proteins in the complex protein mixture.

As discussed above, neither Aebersold nor Cravatt, taken alone or in combination, are capable of rendering obvious the present invention, as defined in the amended claims. Indeed, as acknowledged by the Examiner, "Aebersold et al and Cravatt et al differ from the instant invention in failing to teach prior to the proteolyzing step, the [sp—that] one or more active target protein[(s)] bound to the probe are bound to a solid support." (See page 5, lines 17-19 of the Office Action). Further reliance on Little is unable to cure the deficiencies of the combination of Aebersold and Cravatt, as Little does not address any of the acknowledged limitations of Aebersold or Cravatt, as discussed in detail above.

Accordingly, as described herein, the present invention distinguishes over the prior art in numerous ways, including, for example,

(1) the combination of Aebersold and Cravatt does not teach the use of activity based probes wherein digestion of the protein sample is done prior to separation;

(2) the present invention employs a single activity based probe, rather than sets of isotopically labeled probes; and

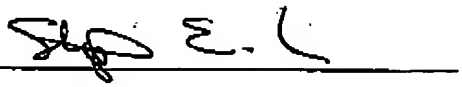
(3) prior to the present invention, the belief was that a single peptide would not provide accurate identification of proteins.

Therefore, in view of the telephone interview, the exhibits submitted herewith, the amendments and remarks presented herein and the remarks and amendments of record, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) as allegedly being unpatentable over Aebersold et al. and Cravatt and further in view of Little are respectfully requested.

In view of the above remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any issues remain to be resolved in view of this communication, the Examiner is invited to contact the undersigned by telephone so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date April 18, 2006

By 

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Attachment: Exhibits I-IV

Exhibit I: Patricelli Protein Analysis; (10/087,602)

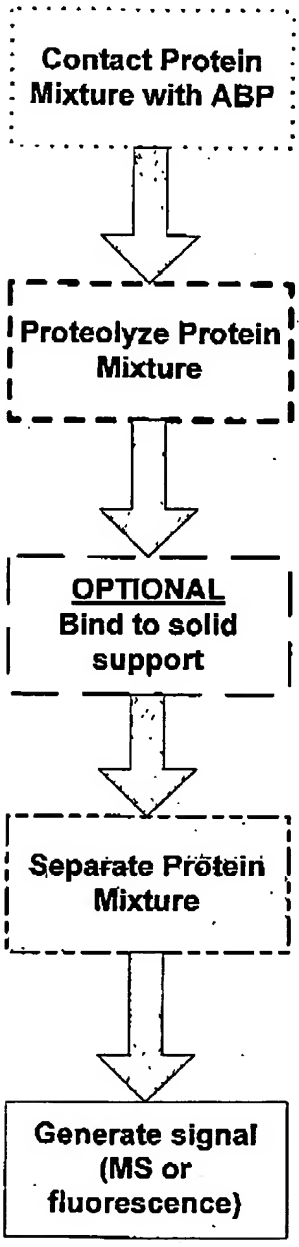

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Exhibit II: Aebersold Protein Analysis; 2002/0076739 (09/839,884)

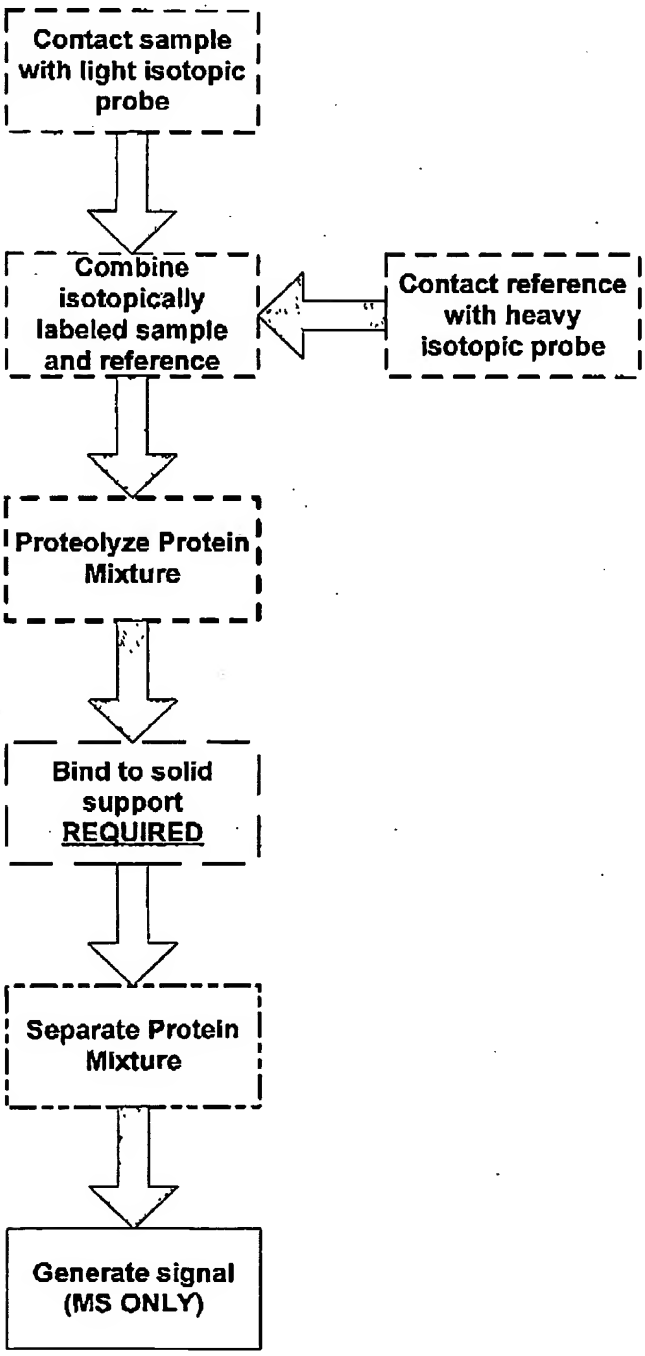

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Exhibit III: Cravatt (Single Probe) Protein Analysis; 2002/0045194 (09/738,954)

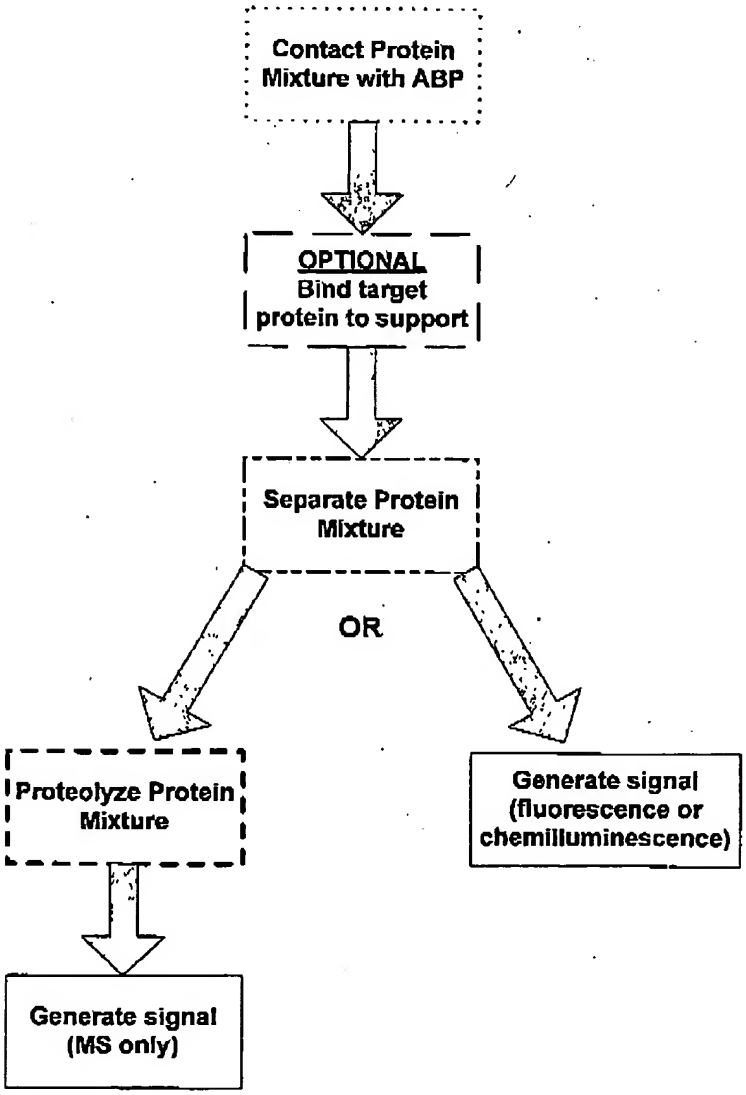
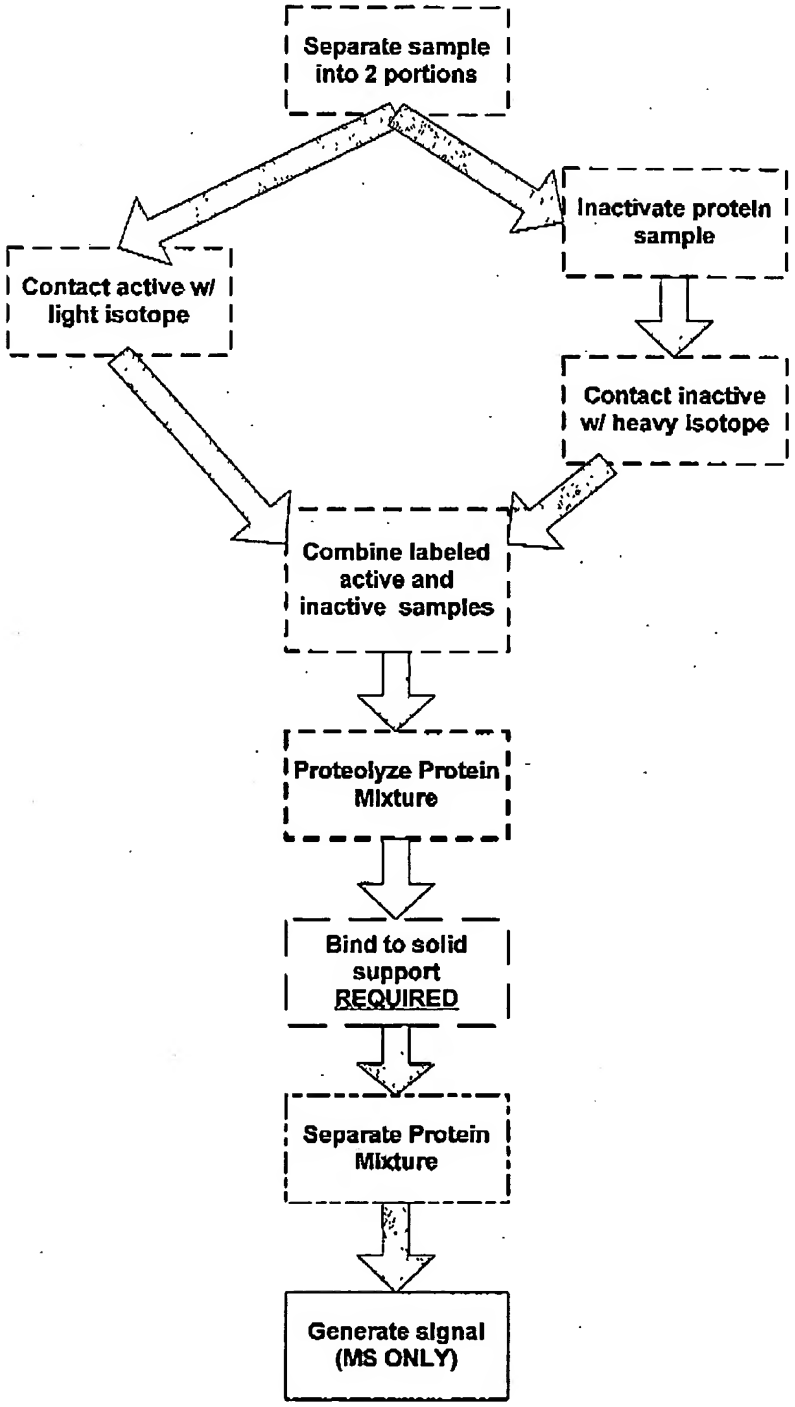


Exhibit IV: Cravatt (Isotopic Probes) Protein Analysis; 2002/0045194 (09/738,954)



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